To examine whether serum levels of soluble interleukin-2 receptor (sIL-2R) is a good marker of acute graft-versus-host disease (aGVHD), they were measured in 37 patients receiving HLA-haploidentical bone marrow transplantation (BMT). Grafts were from HLA-haploidentical relatives. Conditioning regimen was myeloablative including cyclophosphamide and toral body irradiation. GVHD prophylaxis included tacrolimus (TAC)/solumedrol (mPSL)/methotrexate (MTX) (n = 11) and tacrolimus (TAC)/solumedrol (mPSL)/methotrexate (MTX)/mycophenolate mofetile (MMF) (n = 27). Seventeen patients developed aGVHD (Grade I, 9; Grade II, 5; Grade III, 3) after BMT. There was a significant correlation between occurrence of a GVHD and the maximal serum level of sIL-2R. For 11 patients receiving GVHD prophylaxis with TAC/mPSL/MTX, aGVHD occurred in 7 patients (63%). The mean maximal level of sIL-2R (±SE) in 7 patients with a GVHD was 7086 (±1066) and that in 4 patients without aGVHD was 2770 (±450). For patients with GVHD prophylaxis with TAC/mPSL/MTX/MMF, aGVHD occurred in 10 patients (37%). The mean maximal level of sIL-2R in 10 patients with aGVHD was 3944 (±655) and that in 17 patients without aGVHD was 2566 (±358). These data suggest that serum levels of sIL-2R are useful for predicting the occurrence of aGVHD after HLA-haploidentical BMT.

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EVALUATION FOR T CELL POPULATIONS AND CYTOKINE SHIFTS IN NEWLY DIAGNOSED PEDIATRIC CHRONIC GRAFT-VERSUS-HOST DISEASE

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Little is known regarding the immunopathogenesis of chronic graft-versus-host disease (cGvHD) in children. From 2001 through 2005, the Children's Oncology Group (COG) performed a randomized, double blinded, placebo controlled phase three study for newly diagnosed extensive cGvHD in children and adolescents, ASCT0031. Based on murine model data and limited human studies, we hypothesized that cGvHD would be characterized by a Th1/Tc1 shift in T cell activation. Correlative immunologic studies were performed on patients at time of enrollment to evaluate for immune cell populations and T cell function, including cytokine production. Twenty-seven control patients without evidence of cGvHD had peripheral blood collected at 3, 6, 9, and 12 months post hematopoietic stem cell transplant (HSCT) and were used as time matched controls. Fifty-four patients with newly diagnosed extensive cGvHD were evaluated and divided as early cGvHD (3–5 months post HSCT; N = 20) or late-onset cGvHD (≥6 months post HSCT; N = 34). Significance was determined by either a 50% increase or decrease of the mean value compared to control with a P value ≤ .05. In the early-onset cGvHD patients, the total number of CD3+, CD4+, and CD8+ T cell subsets were all lower relative to non-cGVHD controls, although none were significantly different. In the late onset cGVHD patients, a non-statistically significant increase in CD4+ and CD8+ T cell populations were noted compared to time-matched controls. Functional evaluations for cytokine production after in vitro PMA/Ionomycin stimulation (intra-cytoplasmic staining by FACS) revealed no significant difference in either Th1 cytokine production (IFNγ) by CD4+ or CD8+ T cells with early-onset (P = .82, 0.81, respectively) or late onset cGVHD (P = .40, P = .33, respectively). Evaluation for a Th2/Tc2 shift revealed that IL-4 in either CD4+ or CD8+ T cells at early-onset cGvHD (P = .35, 0.11, respectively) or late-onset cGvHD (P = .54, P = .64, respectively) was not different. Thus, we were unable to demonstrate either a Th1/Tc1 or Th2/Tc2 shift associated with newly diagnosed cGvHD in children. These results may be different to previous adult HSCT studies in that there may be a difference in the biology of chronic GvHD in younger HSCT recipients or that these studies were limited to evaluation at the time of presentation of disease.

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BRONCHIOLITIS OBLITERANS AND BRONCHIOLITIS OBLITERANS ORGANIZING PNEUMONIA IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT: AN INSTITUTIONAL EXPERIENCE

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Bronchiolitis obliterans (BO) and bronchiolitis obliterans organizing pneumonia (BOOP) are rare, well-recognized complications of allogeneic stem cell transplant (SCT). Both BO and BOOP are associated with high morbidity and mortality in adult patients. However, in the pediatric population, the incidence and morbidity of these complications have not been well described. We report our institutional experience of BO and BOOP in 449 pediatric allogeneic SCT patients between January 1, 1993 and December 31, 2004. The source of stem cells in this population was as follows: 200 unrelated donors, 198 siblings, 42 parents, 5 extended family members, and 4 half-siblings. A total of 18 patients (4%) developed BO (n = 11) or BOOP (n = 7) during this time. The diagnosis of BO was based on pulmonary function test (PFT) abnormalities (decline in FEV1 ≥20% of baseline or FEV1/FVC <70%) or characteristic histologic changes on lung biopsy. BOOP was diagnosed by pathology only. The most common conditioning regimen was cyclophosphamide (Cy) and total body irradiation (TBI), used in 12 patients. Cytarabine/Cy/TBI was used in 3 cases, Busulfan/Cy in 2, and 1 patient received Etoposide/Cy. All but 2 patients received bone marrow as a stem cell source; the remaining patients received peripheral blood stem cells. Three of the patients received stem cells from fully matched related donors; 6 of the donors were related, but matched at less than 6 of the 6 typed HLA loci. Nine patients received fully matched unrelated donor transplants. The median time to diagnosis of BO or BOOP was 329 days from stem cell infusion. Ten patients had abnormal computed tomography scans at diagnosis. Six patients were diagnosed by PFTs alone, 9 by pathology alone, and 3 by PFTs and pathology. All patients were treated with immunosuppression: 16 patients received corticosteroids, 15 patients received calcineurin inhibitors, and 2 patients received mycophenolate mofetil. Azithromycin was used in 3 patients for anti-inflammatory effects. Four patients were treated with other medication regimens. One patient (6%) experienced complete resolution of pulmonary disease. Three patients (17%) achieved partial resolution. Four patients (22%) had progressive disease. Ten patients (56%) died; 7 of pulmonary disease and 3 of unrelated causes. BO and BOOP, while uncommon, are associated with considerable morbidity and mortality in pediatric SCT, and new therapeutic modalities are needed to improve the outcome of these patients.

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CELLCEPT/CYCLOSPORINE AS PROPHYLAXIS AGAINST GRAFT- VERSUS-HOST DISEASE IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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Traditionally, pediatric patients are prophylaxed against acute graft-versus-host disease (GvHD) with cyclosporine combined with either methotrexate Mtx) or methylprednisolone (Pred). Mtx worsens the severity of mucositis and renal insufficiency while pred causes muscle wasting, hypertension and increased infections. Cellcept (myophenolic acid) is a better tolerated, less toxic agent with synergistic immunosuppressive effects when combined with cyclosporine or tacrolimus. We explored its use in combination with cyclosporine in 21 pediatric patients undergoing allogeneic transplantation. Patients with both malignant (n = 7) and non-malignant (n = 14) conditions ranging in age from 2 weeks to 15 years were transplanted after myeloablative preparative regimens with either related bone marrow or cord blood (n = 7) or unrelated cord blood (n = 14), using cellcept and cyclosporine for GVHD prophylaxis. Cellcept and cyclosporine were administered intravenously through the first 40-60 days post transplant using cellcept at a dose of 15mg/kg/dose IV q8H beginning on day -2 or -3. The patient then transitioned to oral therapy at the same dose and

schedule. There were no acute toxicities attributable to cellcept observed in patients on IV therapy. All patients required treatment with antihypertensive therapy(ies). Grossly infection rates did not vary from those previously observed in conventionally treated patients at our center. 18/21 patients engrafted neutrophils between day 9 and 52. Of the three patients who did not engraft, 2 died of infectious complications, one died of VOD and multisystem organ failure. Of the 18 patients, evaluable for GvHD follow-up ranged between 34 and 693 days (median 108 days), 6 patients had 0 grade I, 10 developed grade II, 1 grade III, and 1 grade 4 GVHD. Follow-up is too short to comment on the incidence of chronic GvHD, but to date, 4 of 7 patients followed for >100 days have developed chronic GvHD. While this data is early, it appears that the incidence of both acute and chronic GvHD are increased compared to previously reported series in patients treated with cellcept instead of mtx (related bone marrow transplantation) or pred (unrelated cord blood transplantation). Engraftment in UCBT recipients was not compromised with the use of IV cellcept administered in the peritransplant period. Additional patients will need to be tested with this regimen and longer follow-up is necessary to fully define the risks or benefits of this therapy.

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OUTCOMES ASSOCIATED WITH A STEROID-CONTAINING ACUTE GVHD PROPHYLAXIS REGIMEN FOR MATCHED UNRELATED DONOR (MUD) HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Previously reported randomized trials have yielded differing conclusions about the addition of steroids to GVHD prophylaxis for patients undergoing sibling allogeneic transplant. Prophylaxis with cyclosporine plus methotrexate after MUD transplant has a reported grade 2–4 acute GVHD incidence of 74%. MUD transplantation was initiated at OHSU in 1996 using a 3-drug combination of cyclosporine, methotrexate, and steroids in a single-arm prospective protocol to examine the incidence and severity of GVHD and survival. **Methods:** The analysis included all patients undergoing MUD transplant who received GVHD prophylaxis with cyclosporine 2 mg/kg iv BID from day −2, methotrexate 15 mg/m2 iv on day +1 and 10 mg/m2 iv on days +3 and +6, and methylprednisolone 0.25 mg/kg iv BID beginning on day +7 and tapering at day +28. Patients were stratified by disease risk per CIBMTR classification. Patients with relapse and no GVHD were censored for GVHD outcomes at therapeutic withdrawal of immunosuppression. **Results:** 124 patients received the 3-drug regimen, including 50 with low-risk, 43 with intermediate-risk, and 31 with high-risk disease. One hundred sixteen (94%) of the MUD grafts were matched at 6 out of 6 HLA-antigens. Eight patients were not evaluable for GVHD due to death prior to engraftment. The maximum grade of acute GVHD is shown in Table 1. Sixty-four (55%) had grade 0–1 acute GVHD. Fifty-two (45%) developed grade 2–4 acute GVHD at a median onset of day +29. Twenty-eight (24%) developed grade 3–4 acute GVHD. Patients with low-risk disease had a median survival of 29 months and a 5-year OS of 42%, whereas those with intermediate/high-risk had a median survival of 10 months and a 5-year OS of 23%. The rate of disease relapse was 15% for both the low and intermediate/high-risk groups. Preliminary infection analysis among 64 patients demonstrated an incidence of all infections greater than 70%. **Conclusions:** The observed rate of grade 3–4 acute GVHD using a 3-drug prophylaxis strategy that includes steroids after MUD transplantation was low and delayed in onset when compared to previous trials of 2-drug combinations. The rate of disease relapse was low and the overall survival was comparable to other published strategies. Preliminary analysis suggests a high rate of infections. Additional analyses are planned to explore rates of chronic GVHD, as well as other potential steroid-related events such as hyperglycemia, musculoskeletal complications, alveolar hemorrhage or thrombotic microangiopathy (Table).

Maximum Acute GVHD Grade

	Number	Percent
Grade 0	**40**	**34%**
Grade 1	**24**	**21%**
Grade 2	**24**	**21%**
Grade 3	**15**	**13%**
Grade 4	**13**	**11%**

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FAILURE OF MEMORY T CELLS TO INDUCE GVHD IS A RESULT OF AN ABORTIVE ALLORESPONSE

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We and others previously reported that effector memory T cells (T_{EM}) can not cause graft-versus-host disease (GVHD). In the current study, we further investigated the potential of this approach, the role of central memory T cells (T_{CM}) in GVHD, and the underlying mechanisms. The study was mainly performed in a major histocompatibility complex mismatched model (unprimed C57BL/6 [$H2^b$] to BALB/c [$H2^d$]). Naive T cells ($CD62L^+CD45RB^+CD44^{low}$), memory T cells (all other T cells except naive T cells), T_{CM} (both $CD62L^+CD45RB^-$ and $CD62L^+CD45RB^+CD44^{high}$), and T_{EM} ($CD62L^-$) were obtained by cell sorting. An in vivo T cell titration experiment demonstrated memory T cells are at least 3-log less potent in mediating GVHD as compared with bulk T cells because infusion of as many as 1×10^6 total memory T cells failed to induce GVHD while infusion of as few as 1×10^3 bulk T cells was able to induce GVHD in this model. Because failure of T_{EM} to induce GVHD may be related to their inability to home to secondary lymphoid organs, we next asked whether T_{CM}, which have the ability to home to secondary lymphoid organs, are able to induce GVHD. The results indicated that neither T_{CM} nor T_{EM} was able to induce GVHD even when as many as 1×10^6 cells were given (Table). All T_{CM} and T_{EM} recipients survived more than 60 days. In contrast, all naive T cell recipients died of GVHD within 40 days after transplantation. These data directly demonstrate that similar to T_{EM}, T_{CM} can not induce GVHD, suggesting that there are other mechanisms involved in the inability of memory T cells to induce GVHD. To further understand these mechanisms, we then studied memory T cells' responses to alloantigens in vitro. As expected, memory T cells failed to elicit cytotoxicity and proliferated poorly against alloantigens in standard 5-day mixed lymphocyte culture as compared with both bulk and naive T cells. However, the proliferation response of memory T cells were much more comparable with those of bulk and naive T cells when the culture time was shortened. More strikingly, the frequencies of IL-2 secreting cells measured by 42-hour ELISPOT assay were similar between naive and memory T cells. These data indicate that memory T cells from unprimed animals are able to respond to alloantigens initially, but fail to develop into full potential. The "abortive" immune response, which may be a feature of non-allospecific memory T cells in response to alloantigens, explains why memory T cells can not induce GVHD (Table).

Central Memory T Cells Cannot Cause GVHD

Groups	% Original body weight		60-Day survival
	day +21	day +56	-
TCD BM alone	**96.4%**	**95.8%**	**100%**
Naive	**70.2%**	**N/A (all dead)**	**0%**
Effector memory	**94.2%**	**93.9%**	**100%**
Central memory	**95.6%**	**98.05**	**100%**